studies also reinforce a critical role for donor APCs in the pathogenesis of GVHD.

25

BENZODIAZEPINE-423, AN INHIBITOR OF MITOCHONDRIAL RESPIRATION, CAUSES SELECTIVE APOPTOSIS OF ACTIVATED LYMPHOCYTES AND REVERSES EXPERIMENTAL GVHD WHILE PRESERVING GVL EFFECTS

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Benzodiazepine (Bz)-423 targets activated lymphocytes through the mitochondrial F_1F_0-ATPase, causing loss of mitochondrial membrane potential and apoptosis without affecting resting lymphocytes. We tested Bz-423 in a non-irradiated B6-Ly5.2→B6F2F1 model of GVHD where donor cells were labeled with CFSE to discriminate between activated ($CFSE^{lo}$) and unactivated ($CFSE^{hi}$) $Ly5.2^+$ donor or host $Ly5.1^+$ lymphocytes. Compared to controls, Bz-423 caused loss of mitochondrial membrane polarization within 6 h of delivery as measured by 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6(3)$) staining in both activated donor $CD4^+$ (32.6% vs 23.1%, $p = 0.04$) and $CD8^+$ (34.5% vs 24.4%, $p = 0.04$) T cells but not in unactivated donor or host cells. Loss of mitochondrial membrane potential was followed by selective apoptosis of donor $CD4^+$ (39.1% vs 25.5%, $p = 0.02$) and $CD8^+$ (41.4% vs 25.9%, $p = 0.002$) T cells. Injection of 60 mg/kg Bz-423 3 times weekly beginning 7 d after GVHD induction significantly reduced mortality (50% vs 100%, $p < 0.02$). We next used Bz-423 in a miHA-disparate, $CD8^+$ T cell-mediated model of GVHD (C3H.SW→B6) in which B6 hosts received 9Gy of TBI and injection of 5×10^6 C3H.SW BM and 4×10^6 T cells, again initiating Bz-423 injections 7 d after BMT. The drug significantly reduced GVHD clinical scores and improved survival compared to controls (74% vs 29%, $p \leq 0.02$). Bz-423 also reduced GVHD histologic damage indices in the liver (3.6 vs 11.2, $p < 0.03$) and the GI tract (7.0 vs 15.8, $p < 0.02$). Bz-423 reduced IFN-γ, a known mediator of GVHD, in the serum (8.4 vs 21.7 pg/ml, $p < 0.03$) and decreased IFN-γ^+CD8^+ effector spleen T cells (0.64×10^5 vs 2.2×10^5, $p = 0.008$), but did not impair the lysis of tumor targets by $CD8^+$ T cells ex vivo. We tested Bz-423 next in a GVL model where EL-4 lymphoma cells syngeneic to B6 recipients were injected on the day of BMT. No recipients of syngeneic BMT survived EL-4 challenge (0/12) and no untreated allogeneic BMT survived GVHD (0/12) but 9/14 (64%) of Bz-423 treated allogeneic recipients were alive on day 50 without evidence of lymphoma ($p = 0.003$). We confirmed the effectiveness of Bz-423 in a third model of GVHD to MHC differences (Balb/c→B6) where again GVHD was reduced, survival was improved (58% vs 8%, $p < 0.002$) and GVL effects preserved. We conclude that Bz-423, a first-in-class compound that selectively inhibits mitochondrial respiration and causes apoptosis of activated lymphocytes, can reverse experimental GVHD while preserving beneficial GVL effects.

26

EPITHELIAL APOPTOSIS IN ORAL CHRONIC GVHD IS MEDIATED BY EFFECTOR-MEMORY CD8 CELLS: POTENTIAL ROLE OF IL-15

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Although chronic Graft versus Host Disease (cGVHD) is a major long-term complication of allogeneic hematopoietic stem cell transplantation, little is known of its pathogenesis. As part of an ongoing NCI cGVHD natural history protocol (04-C-0281), patients were evaluated clinically for oral cGVHD parameters and biopsies were collected from buccal mucosa. To assess histologic correlates of the clinical symptoms, we quantified epithelial injury and mononuclear cell infiltration using multi-parameter immunofluorescent staining for active caspase 3 and CD45 and confocal microscopy on paraffin embedded tissues. The numbers of cleaved caspase-3^+ apoptotic cells within the epithelial layer correlated with clinical severity assessed using the oral cGVHD grading scale. We then characterized the cell populations in severely affected tissues. The predominant infiltrating T-cells were $CD3^+CD8^+$ cells expressing TIA-1 and Granzyme B, markers of cytotoxic effectors. Both CD8 and CD4 T-cells expressed T-bet, a transcription factor characteristic of Type I cytokine producing cells. Consistent with this finding, IFN-gamma levels as measured by quantitative PCR in split biopsies positively correlated with the apoptotic index within the affected oral epithelium ($r = 0.68$), with total infiltrating CD8 cells ($r = .46$) and particularly with proliferating CD8 cells as assessed by double CD8/Ki67 staining ($r = 0.70$). Since the infiltrating CD8 cells expressed the markers of effector-memory lymphocytes (CD45RO), we examined the expression of IL-15, a key cytokine in the generation, proliferation and maintenance of effector-memory CD8 cells. We observed elevated IL-15 expression in both the keratinocytes and infiltrating cells in the dermis of affected oral mucosa. Mean fluorescent intensity of IL-15 in the epithelium correlated with levels of granzyme B mRNA ($r = .72$) in affected tissues, consistent with IL-15 involvement in CD8 effector function. We further investigated the balance of effector and regulatory T-cells by immunofluorescent staining for FoxP3. $FoxP3^+$ cells were quantitatively increased in severe oral cGVHD, but the proportion of $FoxP3^+$ Treg cells in the total CD3 population remained constant. Our findings suggest that infiltration of epithelial layers by CD8 T-cells expressing T-Bet and cytotoxic markers are directly involved in disease pathogenesis and that these cells may be supported by elevated IL-15 produced in the cGVHD epithelia.

27

IL-17 IS REQUIRED FOR CD4-MEDIATED GVHD

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Recently a new subset of CD4+ T cells has been characterized, which has been identified as IL-17 producing CD4+ T cells or Th17 cells. Naive T cells exposed to TGF-β and IL-6 differentiate into Th17 cells by activating RORγt and STAT3 transcription factors. Th17 cells produce high levels of proinflammatory cytokines, including IL-17, IL-21 and IL-22. These cells have been implicated in allograft rejection of solid organs and several autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and colitis.

Except for one report demonstrating the presence of Th17 cells in a murine chronic graft versus host disease (GVHD) model, no data have been presented regarding their role in acute GVHD. Moreover, no studies have addressed the functional role of Th17 cells in GVHD. We first performed DNA microarray analyses of the small intestine from mice with GVHD and found increased expression of Th17 related genes, including IL-17A, IL-17 receptor, IL-6 and TGF-β. Using flow cytometric analysis we detected CD4+ IL-17 producing cells of donor origin at early time points (day 10–14) after transplant in the spleen, mesenteric lymph nodes (MLN), axillary lymph nodes and lamina propria in mice with GVHD, but not in non-transplanted mice or transplanted mice without GVHD. We confirmed these findings in two MHC-disparate models, as well as ELISA of the supernatant of in vitro stimulated T cells from the spleen or MLN from mice with GVHD. Interestingly, we observed significant numbers of alloreactive CD4+ T cells, which produce both IFN-γ and IL-17, suggesting that alloreactive T cells in vivo can express both Th1 and Th17 phenotypes simultaneously.

To assess whether Th17 cells are required for the development of acute GVHD, we performed experiments with donor T cells from IL-17 deficient mice. In a series of reproducible experiments with varying doses of selected CD4+ T cells from WT and IL-17-/- donors, we found significantly less GVHD morbidity and mortality in the recipients of IL-17-/- T cells. Finally, preliminary experiments

with CD4+ T cells from RORγt-/- vs WT donors suggest a significant survival benefit for recipients of RORγt-/- T cells. In conclusion, these data suggest that CD4+ Th17 cells contribute to the development of acute GVHD.

28

IL13 +2044 (ARG130GLN) ASSOCIATES WITH ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE FOLLOWING HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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IL-13 is a T-helper 2 (Th2) cytokine that suppresses the cytotoxic action of macrophages and inhibits the production of pro-inflammatory cytokines. A single nucleotide polymorphism (SNP) exists within exon 4 of the *IL13* gene at position +2044 which results in an amino acid substitution (Arg130Gln). The high producer variant (A allele) of this SNP, has been associated with several inflammatory conditions, most notably asthma, and more recently IL-13 mixed lymphocyte supernatant levels have been associated with aGvHD in HSCT. Consequently the role of *IL13* +2044 SNP in HSCT was examined in this investigation.

Polymorphism studies were carried out in a cohort of 923 HSCT recipient and donors from 7 transplant centres across Europe. Genotyping was performed using PCR and RFLP analysis. Univariant (chi-squared) and multivariant (binary logistic regression) analyses were performed to study associations and P values <0.05 were regarded as being statistically significant. Clinical factors such as recipient age, gender mismatch (female donor/male recipient), CMV status, stem cell source and treatment regimens were also included in the analyses.

Multivariant analysis of the whole HSCT cohort demonstrated that patients possessing the A allele were more susceptible to developing severe aGvHD (grades III-IV) (P = 0.028). Patients receiving transplants from donors with the *IL13* +2044 A allele were more at risk of developing cGvHD (P = 0.026). These associations remained significant when the cohort was stratified for transplant type (HLA-matched sibling and matched unrelated donor) and conditioning regimen (T replete vs deplete and reduced vs full intensity conditioning). Significant associations were also observed in a subset of patients diagnosed with CML; HLA-matched sibling patients possessing the *IL13* +2044 A allele were less likely to develop cGvHD, whereas in MUD transplants donor possession of the A allele was a risk for cGvHD. Depending on the subset analysis, clinical factors, particularly peripheral blood stem cell transplants, were significantly associated with GvHD.

To our knowledge this is the first investigation examining the role of *IL13* +2044 SNP in HSCT. The findings are extremely encouraging, indicating that *IL13* +2044 SNP is associated with the development of both aGvHD and cGvHD and consequently could provide key pre-transplant information on GvHD prognosis.

29

ADDITION OF ETANERCEPT TO METHYLPREDNISOLONE AS INITIAL THERAPY FOR ACUTE GVHD RESULTS IN HIGH RESPONSE RATES AND IMPROVED SURVIVAL

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Standard therapy for GVHD, high dose steroids, results in complete responses in only 35% of patients. Because tumor necrosis factor (TNF) is an important effector of GVHD we treated 61 pts with new onset GVHD with methlyprednisolone 2 mg/kg/d plus etanercept, a TNF inhibitor. All pts continued their prophylaxis agent, usually tacrolimus, at therapeutic dosing.

Etanercept was given subcutaneously 2× weekly for 8 wks at a dose of 0.4 mg/kg/dose (max dose 25 mg). The outcomes in these 61 pts were compared to those of 99 contemporaneous pts with GVHD whose initial therapy was steroids alone. Both groups of pts were similar with respect to age, transplant conditioning intensity, donor type, degree of HLA-match, and severity of GVHD at onset. Pts who acheived CR by 4 wks did so without any second line agents. Pts treated with etanercept plus steroids were significantly more likely to achieve a complete response 4 wks later than were pts treated with steroids alone [69% vs 33%, p < 0.0001]. The benefit of etanercept persisted: at 12 wks after initiation of GVHD treatment, 77% of etanercept plus steroids pts had achieved CR compared to 50% of steroids alone pts (p = 0.0009). The superiority of etanercept plus steroids over steroids alone was observed in in all three target organs. Differences in steroid dosing did not account for the difference in CR rate.

There was a trend for better survival at 6 mo following initiation of GVHD treatment for the etanercept treated pts [p = 0.08] which is explained by significantly superior survival in the unrelated donor pts whose GVHD was treated with etanercept. Although related donor pts who were treated with etanercept plus steroids were more likely to be in CR at 4 wks [79% vs 39%, p = 0.001], by 12 weeks nearly equivalent proportions of pts in both groups achieved a CR (etanercept plus steroids, 80%, steroids alone 70%) and 6 mo survival was similar. A greater proportion of unrelated donor pts treated with etanercept were in CR at 4 wks [53% vs 26%, p = 0.0005] but, unlike related donor pts, steroids alone pts who failed to achieve a CR by 4 wks were likely to never achieve CR. This difference in overall CR rate translated into better survival six months later [p = 0.05].

Although not a randomized trial, these data strongly suggest that etanercept plus steroids as initial therapy for acute GVHD results in improved complete response rates compared to steroids alone and may improve survival, especially for unrelated donor transplant pts.

Complete Response Rates at Four Weeks According to Treatment Group

	Steroids alone	Etanercept plus steroids	p value
Overall	**33/99 (33%)**	**42/61 (69%)**	**<0.0001**
Skin	**32/68 (47%)**	**30/37 (81%)**	**0.0008**
Liver	**3/15 (20%)**	**6/9 (67%)**	**0.03**
GI	**21/44 (48%)**	**29/37 (78%)**	**0.005**
Presenting grade			
Grade II	**25/68 (37%)**	**31/40 (77%)**	**0.0001**
Grade III/IV	**8/31 (26%)**	**11/21 (52%)**	**0.05**

30

A FULLY-HUMAN CHIMERIC ANTIGEN RECEPTOR FOR REDIRECTING SPECIFICITY OF T CELLS TO B-LINEAGE TUMORS

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Adoptive therapy after allogeneic hematopoietic stem-cell transplantation with *ex vivo*-expanded donor-derived tumor-specific T cells to augment the graft-versus-leukemia (GVL) effect might be used to reduce the incidence of leukemic relapse without exacerbating graft-versus-host disease. We previously showed that genetically modified peripheral- and umbilical cord blood-derived T cells rendered specific for CD19, a molecule constitutively expressed on B-cell malignancies can augment GVL-effect. The redirected specificity was achieved by electro-transfer of a DNA plasmid coding for the chimeric antigen receptor (CAR) that recognizes CD19 via the scFv of a murine CD19-specific monoclonal antibody (mAb) fused to T-cell activation endodomains. However,